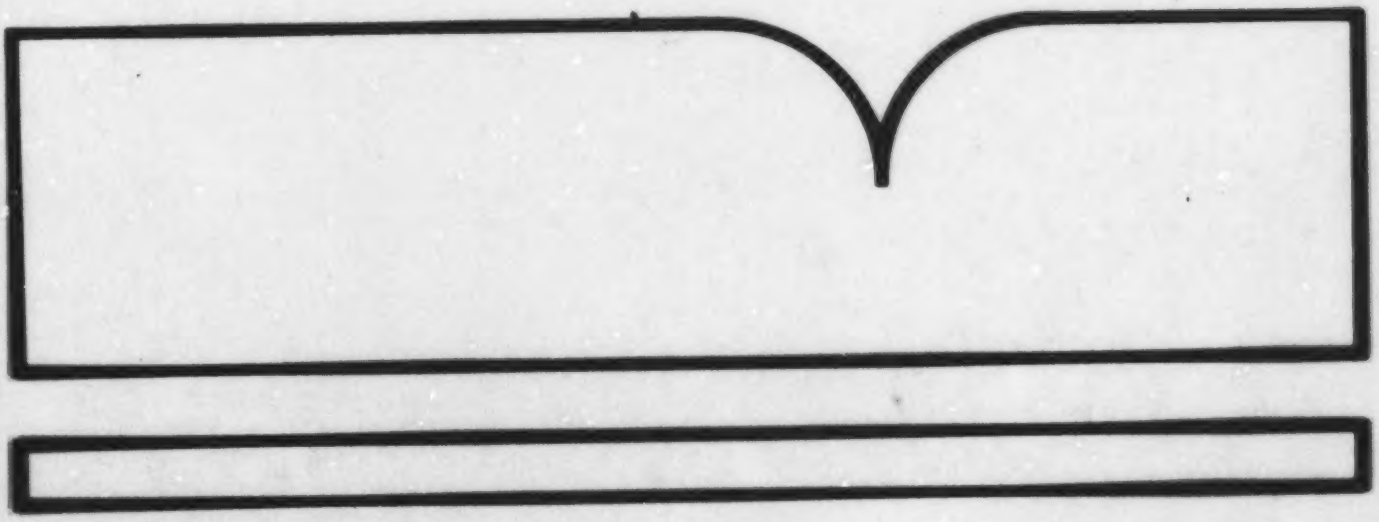
PB86-230877

Toxicity of Fenvalerate to Developing Steelhead Trout Following Continuous or Intermittent Exposure

(U.S.) Corvallis Environmental Research Lab., OR

1985



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1. REPORT NO. EPA/600/J-85/444	2.
3 PB86-230877	
4. TITLE AND SUBTITLE Toxicity of Fenvalerate to Developing Steelhead Trout Following Continuous or Intermittent Exposure (Journal Version)	5. REPORT DATE 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Curtis, L.R., W.K.Seim, and G.A.Chapman	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Oak Creek Laboratory of Biology Dept of Fisheries & Wildlife, OSU Corvallis, OR 97331	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. CR807260-020
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S.Environmental Protection Agency Corvallis, OR 97333	13. TYPE OF REPORT AND PERIOD COVERED Journal Article
	14. SPONSORING AGENCY CODE EPA 600/02

15. SUPPLEMENTARY NOTES
1985. Journal of Toxicology and Environmental Health 15:445-457. Project officer: Gary Chapman FTS 420-4876.

16. ABSTRACT

Environmental toxicant exposures commonly vary in terms of duration and concentration. However, laboratory toxicity tests usually entail continuous exposures to constant concentrations. We compared survival, growth, and toxicant accumulation in early life-stage steelhead trout intermittently or continuously exposed to fenvalerate (FV) for 70 d after fertilization. Acute lethality was assessed in ancillary 96-h LC50 determinations with juvenile fish. Intermittent exposures were daily 4.5-h introductions of toxicant, and continuous exposures were to constant concentrations. All tests were conducted in a flow-through dilution apparatus, and mean concentrations for the entire exposure period were calculated for comparisons between regimens. The respective 96-h LC50 values for intermittently and continuously exposed were 88 and 172 ng/l. In the subchronic study, marked lethality (32%) and reduced terminal weight (50%) were found following exposures to cyclic FV concentrations that yielded an average of 80 ng/l (peak of 461). Continuous exposure to 80 ng FV/l did not affect these parameters. At mean FV concentrations above 20 ng/l, bioaccumulation was greater following intermittent that continuous exposure. Interaction of partitioning and elimination processes may partially explain differences in FV accumulation and subsequent toxicity.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
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18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 15
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

EPA/600/J-85/444
JOURNAL ARTICLE

TOXICITY OF FENVALERATE TO DEVELOPING STEELHEAD TROUT FOLLOWING CONTINUOUS OR INTERMITTENT EXPOSURE

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Environmental toxicant exposures commonly vary in terms of duration and concentration. However, laboratory toxicity tests usually entail continuous exposures to constant concentrations. We compared survival, growth, and toxicant accumulation in early life-stage steelhead trout intermittently or continuously exposed to fenvalerate (FV) for 70 d after fertilization. Acute lethality was assessed in ancillary 96-h LC50 determinations with juvenile fish. Intermittent exposures were daily 4.5-h introductions of toxicant, and continuous exposures were to constant concentrations. All tests were conducted in a flow-through dilution apparatus, and mean concentrations for the entire exposure period were calculated for comparisons between regimens. The respective 96-h LC50 values for intermittently and continuously exposed were 88 and 172 ng/l. In the subchronic study, marked lethality (32%) and reduced terminal weight (50%) were found following exposures to cyclic FV concentrations that yielded an average of 80 ng/l (peak of 461). Continuous exposure to 80 ng FV/l did not affect these parameters. At mean FV concentrations above 20 ng/l, bioaccumulation was greater following intermittent than continuous exposure. Interaction of partitioning and elimination processes may partially explain differences in FV accumulation and subsequent toxicity.

INTRODUCTION

Most data describing subchronic effects of toxicants on experimental animals represent several exposure concentrations and one dosing schedule. In tests with aquatic organisms, conditions of continuous exposure to essentially constant concentrations predominate. Criteria or

This research was supported by cooperative agreement CR807260-020 from the U. S. Environmental Protection Agency.

Preliminary results of this research were presented at the 1983 Annual Meeting of the Society of Toxicology, Las Vegas, Nevada.

We extend our thanks for technical assistance to Susan Glenn and Mike Becerra. Fenvalerate used in these studies was donated by Shell Development Company (Modesto, California).

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Journal of Toxicology and Environmental Health, 15:445-457, 1985
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standards developed from such data may not be appropriate for extrapolation to environmental situations where exposures occur intermittently. Transient elevations of contaminant concentrations result from variable introduction due to cyclic industrial processes, aerial chemical application, and contaminated precipitation. Earlier work showed that repetitive intermittent exposures to metal concentrations that produced no effect in acute continuous mortality tests could be toxic to *Daphnia pulex* (Ingersolland and Winner, 1982). Seim et al. (1984) demonstrated that subchronic exposure of early life-stage steelhead trout to copper (expressed as mean concentrations) was much more damaging when the delivery of toxicant was cyclic rather than continuous. This work was conducted to ascertain whether similar results would be obtained when a chemical with very different toxicological and physiocochemical properties was tested.

Fenvalerate (FV), a synthetic organic insecticide, was chosen for study (Fig. 1). Duration of intermittent exposures, life stages, strain of fish tested, experimental temperature and water, and as many other factors as feasible were replicated from an earlier study with copper (Seim et al., 1984) to facilitate comparison of results. Fenvalerate is one of a family of recently developed insecticides classed as synthetic pyrethroids. These agents have high insecticidal activity, exhibit very low avian and mammalian toxicity, but are very toxic to fish (Casida et al., 1983; Miyamoto, 1976). The physicochemical properties of these esters and their persistence in the environment are modified by terminal substitutions in their side chains (Casida, 1980; Elliott, 1976). Incorporation of a cyano group, phenyl ring, or chlorine enhances stability, while presence of the latter two increases lipophilicity. Fenvalerate possesses all of these substitutions (Fig. 1). We selected FV for study because of its (1) chemical stability, (2) extreme potency in terms of toxicity to fish, and (3) representativeness of an environmentally significant class of chemicals.

Selection of a meaningful expression for comparison of continuous and intermittent toxicant exposures was carefully considered. The 24-h mean and maximum (peak) toxicant concentration were both proposed as a basis for standards designed to protect aquatic organisms (Anonymous, 1980). Coffin et al. (1977) used cumulative total concentrations to compare toxicity of intermittent and continuous inhalation exposures of mice to nitrogen dioxide, sulfur dioxide, and benzene. Calculation of

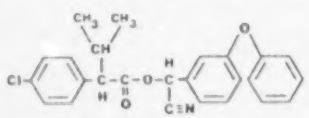

FIGURE 1. Chemical structure of fenvalerate (MW 420). From Chemical Abstracts Service no. 51630-58-1.

exposure units (μg toxicant/l•h exposure) was previously considered by this group (Seim et al., 1984). As was true for cumulative total concentration, exposure units were directly proportional to means. We therefore selected mean concentrations to compare intermittent with continuous FV exposures.

METHODS

Experimental Animals

Steelhead trout *(Salmo gairdneri)* eggs and sperm were obtained from the Alsea Fish Hatchery (Oregon Department of Fish and Wildlife). Eggs taken from 2 females were fertilized with sperm from 3 males and allowed to stand in water for 1 h prior to transport to the laboratory. Upon arrival, 60-ml samples (approximately 240 eggs) were placed as a single layer in each of 20 incubation chambers. Six days later, on February 24, 1982, exposures to continuous or intermittent toxicant flows began. The experiment was concluded after 70 d of exposure.

About 8 wk after fertilization, 120 randomly selected fish from each treatment were placed in aquaria and fed Oregon Test Diet (Sinnhuber et al., 1977) at 12% body weight/d. The ration supplied to each group was adjusted for fish wet weights determined every 2 wk. No attempt was made to quantify the food not consumed, but the aquaria were cleaned daily to remove uneaten food.

Exposure Apparatus

A flow-through dilution apparatus was constructed as a modification of the design of Chadwick et al. (1972). Well water flowed through plastic float valves into two connected headboxes, where the temperature was maintained at 12 ± 0.5° C by thermostatically controlled, stainless-steel immersion heaters. Room temperature was also held at 12° C. Flow rates of dilution water and stock toxicant solution were measured daily, and necessary adjustments were made. Because of the low water solubility of FV (about 2-4 μg/l at 12° C in our system), the toxicant was first dissolved in acetone and coated onto mixed fine gravel. After the solvent had evaporated, the coated gravel was placed in a glass column (5 × 75 cm). Water was then recirculated through the column from the stock aquaria that supplied toxicant to the diluter, via a gravity-flow delivery manifold (Gingerich et al., 1979). A timer-controlled pump regulated inflow of stock solution to the delivery manifold that served intermittent exposure chambers. The timer was set to introduce a single daily 4.5-h pulse into the test system. The total flow of dilution water plus stock toxicant solution to each incubation chamber was approximately 400 ml/min.

Two incubation chambers for each concentration were constructed from two-piece, polypropylene Buchner funnels firmly seated in wooden cross-supports. The upper compartment, which housed the

embryos, was an 18-cm-diameter, 15-cm-high cylinder with a perforated bottom and open top. The lower compartment's spout was fitted with polyvinyl chloride (PVC, Tygon) tubing to permit inflow of water from the delivery manifold. Water flowed up through the test exposure chamber and was discharged through a number of small holes drilled just below the top. Each incubator was covered with a plate of untreated wood to exclude light. The calculated 90% replacement time in the incubators was 15 min (Sprague, 1969).

When control fish began active swimming and feeding, 120 larvae from each treatment (60 from each duplicate funnel, fewer where mortality was high) were transferred to an individual 30-l glass aquarium for the remaining 27 d of the experiment. The 90% replacement time for aquaria was 90 min, with an exchange flow of 800 ml/min (Sprague, 1969).

Exposure and Sampling Procedures

Toxicant introduction began 6 d after fertilization. The FV concentration of the stock solution was measured twice weekly at different times in the exposure cycle throughout the experiment to account for variable demand on the saturator incurred by intermittent dosing. Weekly samples were also collected from each exposure chamber. The time-course of concetration during intermittent exposure was measured twice for each intermittent treatment (once in each funnel and aquarium), and the means were used for drawing time/concentration curves (Fig. 2). Ten samples were collected over a 7-h period: immediately prior to toxicant introduction, at 0.1, 0.25, 1, 2, and 4.5 h into the pulse, and at 0.5, 1.5, 2.0, and 2.5 h after the pulse terminated. Areas under the curves were measured by planimetry. Areas under the time/concentration curves, in units of $\mu g/l \cdot h$ exposure, were divided by 24 h to estimate the daily mean concentrations.

Fenvalerate was tested at continuously maintained concentrations, which averaged 18 ± 8, 80 ± 17, 135 ± 24, and 505 ± 150 ng/l (means $\pm$ SE) over the course of the study. When introduced daily for intermittent 4.5-h periods, concentrations above background persisted for about 7 h. The shapes of the time/concentration curves (Fig. 2) were similar to those published previously (Seim et al., 1984). For intermittent exposures, the mean concentrations calculated for the entire study were 5, 20, 40, and 80 ng/l, with the corresponding mean daily peaks at 29 ± 9, 93 ± 8, 173 ± 31, 461 ± 67 ng/l.

Daily removal of any discernibly dead embryos and unfertilized eggs began 5 d following toxicant introduction. Death of embryos was determined by the "cloudy" appearance associated with water uptake by nonviable eggs (Hayes, 1949). Cessation of spontaneous body movement and failure to respond to touch were criteria of death for larval animals. Live fish were sampled for determination of dry weight when

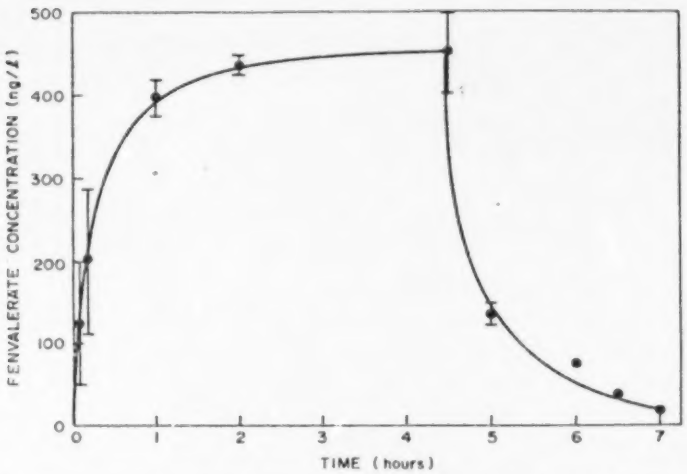

FIGURE 2. Time course of total fenvalerate concentration for the highest intermittent exposure. Results are means of 2 independent sampling sequences, except the 6-, 6.5-, and 7-h points, which were done once.

50% of the eggs were hatched (at about 5 wk postfertilization) and at 2-wk intervals thereafter until the experiment terminated. Twenty fish from each concentration were overdosed with MS-222 solution, the remaining yolk was removed, and individual animals were placed in preweighed aluminum pans. Following drying for 96 h at 70° C, the fish were cooled in a dessicator and weighed on an analytical balance. Samples for residue analysis were taken on the final day of the experiment.

Chemical Analysis

Water samples were removed weekly from a control chamber and a chamber at the highest exposure concentration. Dissolved oxygen remained near air saturation throughout the study (range of 9.2-10.4 mg/l). Hardness averaged 129.2 mg/l, and alkalinity 127.7 mg/l as calcium carbonate. The pH of control water varied from 7.8 to 8.2 over the 70-d study. The pH of water in the control tank differed from that of any treatment tank by less than 0.1 unit throughout the study.

Fenvalerate concentrations in water and tissue (whole fish) were determined on a Hewlett Packard model 5700A gas chromatograph equipped with an electron-capture detector. Water samples (250 ml) were extracted twice with hexane (50 ml) by shaking for 4 min in a separatory funnel. Hexane extracts were concentrated by evaporation to 15 ml under a nitrogen stream. Recovery from FV-spiked water averaged 90%. No significant degradation of FV was apparent in the stock solution over the course of the study. Fenvalerate residues in whole fish were determined by methods described by Analytical Chemistry Department,

Shell Development Co., Modesto (1983). FV recovery from spiked tissue samples averaged 83%. FV was chromatographed under the following conditions: a 1100 × 4 mm (ID) glass column packed with 3% OV 101 on Chromasorb W (80-100 mesh); detector, injector and oven temperatures of 200, 300, and 265° C, respectively; carrier gas, argon-methane (95:5, v/v), 30 ml/min.

Acute Mortality

The 96-h LC50 was determined for a group of control fish following termination of the embryo-larval test. The fish were held in 30-l aquaria receiving a water flow rate of 400 ml/min. Analysis of duplicate water samples indicated continuous exposure concentrations of 10±15, 13±16, 237±25, 430±25, and 610±70 ng/l; for the intermittent 4.5-h/d exposure, peak concentrations were 387±61, 460±40, 494±37, and 666±37 ng/l (corresponding means of 73, 86, 93, and 125 ng/l). Ten fish (2-3 g wet weight) were exposed to each concentration.

Data Analysis

Mortality data were analyzed by the "trimmed" Spearman-Karber method (Hamilton et al., 1977). One-way analysis of variance followed by the Student-Newman-Keuls test was applied to the weight data (Sokal and Rohlf, 1969). Weights for intermittent and continuous exposures at a mean of 80 ng/l were compared with the *t*-test. Residue determinations were analyzed by analysis of variance and nonlinear regression performed by the method of least squares. Significance was set at $p < 0.05$.

RESULTS

Dilutor Performance

The saturator delivered a stock FV solution of 1.61 ± 0.23 μg/l (mean ± SEM, $n = 24$) to the dilutor manifold over the course of the study. The extreme hydrophobicity of the compound and flow variation through the system demanded by intermittent dosing contributed to fluctuation in concentration. To compensate for variation in saturator performance, results were expressed as means of concentrations measured in exposure chambers at different times in the daily cycle. Agreement in nominal and measured concentrations in individual chambers was complicated by FV's lipophilicity. Water samples from exposure chambers that contained excessively high FV concentrations (i.e., 4-10 times nominal) were encountered around the time of hatching, but not before. No parallel increase in concentration of stock solution occurred. The incidence of this phenomenon declined from around 50% during hatch to 0% following transfer to aquaria and initiation of feeding. Further, such spurious results were not obtained in the acute toxicity test (namely, 96-h LC50). The occurrence of organic debris in incubation chambers

TABLE 1. Time Course for Responses of Developing Steelhead Trout to Continuous and Intermittent Fenvalerate Exposures

Exposure	Concentration (ng/l)		Percent survival and mean dry weight[a] (in parentheses) at days postfertilization							
	Mean	Peak	14	28	35	42	49	56	63	70
Continuous	0	(control)	98	93	93 (25.0 ± 0.9)	93	93 (55.0 ± 0.7)	93	92 (78.8 ± 2.2)	91 99.6 ± 3.2)
	18		98	95	95 (23.4 ± 0.8)	95	94 (53.7 ± 0.8)	93	93 (76.6 ± 2.6)	93 (99.9 ± 4.0)
	80		98	93	93 (22.0 ± 1.4)	92	91 (52.8 ± 0.8)	90	90 (71.3 ± 2.7)[b]	90 (88.3 ± 2.3)[b]
	135		96	92	89 (14.2 ± 0.9)[b]	79	36 (35.0 ± 0.7)[b]	23	12 (26.9 ± 1.2)[b]	8 (23.8 ± 9.2)[b]
	505		96	94	89 (9.5 ± 0.6)[b]	8	0	0	0	0
Intermittent	5	18	98	96	95 (25.7 ± 1.0)	94	94 (53.3 ± 0.6)	94	94 (81.8 ± 2.0)	94 (108.8 ± 3.6)
	20	51	99	95	94 (23.8 ± 1.0)	92	91 52.9 ± 0.7)	91	91 (75.2 ± 2.2)	91 (95.9 ± 5.1)
	40	148	98	95	94 (22.5 ± 0.7)	85	85 (51.4 ± 0.6)	84	83 (70.7 ± 2.0)	82 (78.4 ± 2.5)[b]
	80	403	97	95	95 (24.9 ± 0.7)	93	91 (45.3 ± 0.9)[c]	81	71 (48.0 ± 1.6)[c]	68 (50.6 ± 2.7)[c]

[a] Results are means ± SEM in mg for 20 fish.
[b] Significantly different from control at $p < 0.05$.
[c] Significantly different from control and equivalent mean continuous concentration.

was readily observable during the time of hatching. Such material (including fragments of chorion and lipid droplets from yolk) undoubtedly contaminated water samples taken. Information on FV's partitioning characteristics (Schimmel et al., 1983) suggested that FV accumulated on suspended particulates of biological origin. We therefore eliminated determinations from our data that exceeded nominal by a factor of four or greater.

Embryo/Larval Mortality

Survival of steelhead embryos and alevins continuously exposed to FV at concentrations of 80 ng/l and less was above 90% (Table 1). Survival was less than 10% at 135 ng/l with most of the mortality occurring after 8 wk of exposure. Steelhead exposed to FV at 505 ng/l had all died before termination of the experiment, with most mortality occurring after 7 wk of exposure (Table 1). Animals exposed intermittently at peak concentrations of 200 ng/l (mean concentration of 40 ng/l) or less exhibited survival rates within 8% of control (Table 1). Of the steelhead exposed to a peak of 590 ng/l (mean of 80), 68% survived.

At a mean of about 80 ng FV/l, survival was 22% greater for developing steelhead exposed continuously rather than intermittently (Table 1). Thus for equal mean concentrations, embryos and larvae exposed to daily toxicant pulses of about 7 h duration exhibited greater mortality than those exposed continuously. When expressed as maximum or peak concentrations, intermittently exposed steelhead had greater survival than those continuously exposed, since they were exposed less than one-third the time (data not shown).

Fish Size

Rates of yolk absorption and appetite were severely reduced at high toxicant concentrations. Mean dry weights of FV-exposed steelhead were not significantly affected until 3 wk after hatching (Table 1). Shortly thereafter, all fish in the group with the highest level of continuous exposure died. Concentration-dependent reduction of growth appeared in other groups as the test progressed (Table 1). By the end of the study, inhibition of growth was apparent at the two highest intermittent concentrations and the two highest remaining continuous concentrations. Measurements of unconsumed ration were not attempted due to the small particle size, but residual food was regularly observed only in the tanks of fish where growth was impaired. Final dry weights demonstrated a greater reduction in size for fish exposed to FV intermittently than for those exposed continuously (Fig. 3). The size difference appeared greatest at 80 ng/l (the highest intermittent exposure).

Fenvalerate Residues

Whole-animal FV concentrations of fish sampled at the end of the

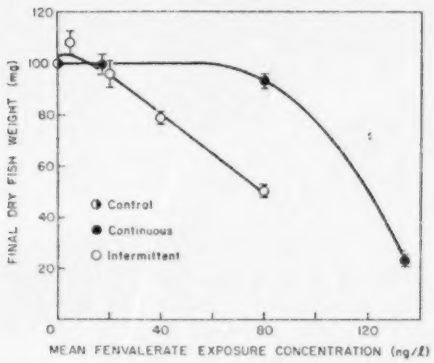

FIGURE 3. Comparison of the mean (±SE) dry weight of steelhead alevins (n=20) following 70 d of intermittent or continuous fenvalerate exposure. Concentrations are expressed in terms of the mean fenvalerate concentrations measured in exposure water throughout the experiment.

study increased with exposure concentration in an exponential manner (Fig. 4). At a low mean exposure concentration (i.e., approximately 20 ng/l), continuous treatment yielded a higher residue content than intermittent exposure, while at a higher mean concentration (i.e., 80 ng/l), the reverse occurred. Bioaccumulation ratios (concentration in tissue divided by mean water concentration) also tended to increase with exposure concentration. Continuous treatment at a mean of approximately 20 ng/l produced higher bioaccumulation ratios than equivalent intermittent treatment, while at 80 ng/l, mean exposure, the opposite resulted.

Acute Mortality

The 96-h LC50 determined for fish from the same group was 172 ng FV/l for continuous exposure (95% confidence levels of 138-215). Intermittent-exposure LC50 test results yielded a value of 88 ng FV/l (95% confidence limits of 84-92). These results support the implication of the embryo-larval test. Intermittent exposure to FV was more deleterious than continuous exposure at equivalent mean concentrations.

DISCUSSION

Exposure of fish to toxicants in the embryo-larval and early juvenile stages is perhaps the most sensitive method for establishing water quality criteria (McKim, 1977). The results of our embryo-larval and acute mortality tests indicated intermittent FV exposures were more toxic than continuous FV exposures when mean concentrations were compared. This agreed quite well with our earlier work with similar exposures to copper in the same strain and life stages of trout (Seim et

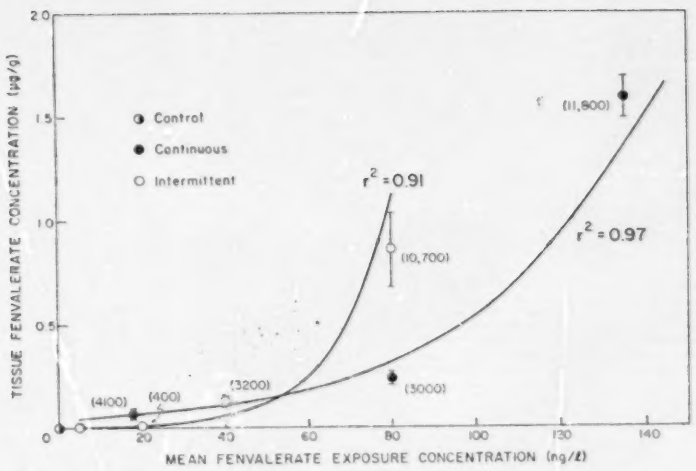

FIGURE 4. Arithmetic plot of mean exposure water concentration versus whole fish fenvalerate concentration (mean ± SE) after approximately 10 wk of treatment. Each point represents three or four individual animals. The fitted lines describe an exponential relationship with the associated coefficients of determination shown. The bioaccumulation ratio (concentration in tissue divided by water concentration) for each treatment is shown in parentheses.

al., 1984). These results indicated continuous exposure tests can underestimate chemical toxicity in intermittent exposure situations.

Fenvalerate-induced mortality became apparent after 6 wk at 505 ng/l in continuously exposed fish and rapidly eliminated that group, while a similar but slower progression occurred at 135 ng/l (Table 1). Marked mortality was not seen at other continuous concentrations. Marked mortality in intermittently exposed animals was seen only after about 10 wk at the 80-ng/l mean concentration (Table 1). Examination of a plot of percentage mortality versus FV concentration did not permit estimation of 50% lethal concentrations for intermittent exposures in the embryo-larval test, but a value of 101 ng FV/l (95% confidence limits of 95-108) was determined for continuous exposure. Data from our 96-h LC50 test also showed that intermittent exposure produced mortality at a lower mean concentration than did continuous exposures. In fact, the former appeared about twice as potent.

Onset of effects on fish size occurred before the initiation of feeding (Table 1). Thus, FV's influence on this parameter cannot be solely attributed to decreased appetite. At a mean of 80 ng FV/l, the size of continuously exposed fish was only marginally affected, while that of intermittently exposed animals was reduced by half (Fig. 3). Sublethal effects on growth therefore support our interpretation of the mortality data: intermittent was more toxic than continuous FV exposure at equivalent mean concentrations.

As in our work with copper (Seim et al., 1984), toxicant bioaccumulation at least partially explained differences in toxicity at equivalent mean exposure concentrations. At mean intermittent exposures of 5 and 20 ng/l, fish may have eliminated a significant amount of absorbed FV between toxicant pulses. The elimination half-life for a related compound (permethrin) in juvenile trout was in hours (Glickman et al., 1981). Glickman et al. also showed the predominant process involved oxidative metabolism, glucuronidation, and biliary excretion. In a study with an invertebrate (oysters), FV depuration was rapid: tissue concentrations fell to the detection limit within 3 d of transfer from contaminated to clean water (Schimmel et al., 1983). At higher mean intermittent concentrations (40 and 80 ng/l) and in all continuous treatments, the depuration rate was apparently insufficient to preclude marked bioaccumulation (Fig. 4). Fenvalerate's high lipophilicity, as indicated by a log P of 6.2 (where P = octanol:water partition coefficient) (Schimmel et al., 1983), accounted for these observations. Simple partitioning of FV into organisms and a saturable elimination process can explain the higher residues found in fish intermittently treated than in those exposed continuously at equivalent mean concentrations. The maximum concentrations reached during intermittent exposures were three to five times higher than those of the equivalent continuous ones. The FV residues at the mean 80-ng/l exposure approximated a 3 to 1 (intermittent to continuous) ratio.

The concentration-dependent increases in bioaccumulation factors (Fig. 4) were consistent with saturation of the elimination process. Also, the small body size attained by animals at the highest toxicant concentrations (Fig. 3) probably contributed to elevated tissue concentrations. Spehar et al. (1982) also reported that FV bioaccumulation ratios increased with exposure concentration in fathead minnows.

Factors other than enhanced bioaccumulation may be involved with greater FV toxicity in our intermittent versus continuous exposures. Kumaraguru et al., (1982) reported the gill as a "target organ" for synthetic pyrethroid toxicity in fish, regardless of whether exposure was via water or diet. Distortion of secondary lamellae, including hyperplasia, and separation and sloughing of epithelial layers were noted. No changes in kidney or liver were reported. Occurrence of elevated concentrations during intermittent dosing may have exceeded a threshold above which FV acted as a contact poison. Time between doses may have been inadequate for recovery.

Understanding interactions between time and concentration components of toxicant exposure has wide relevance in toxicology. Work with laboratory rodents (Coffin et al., 1977); Gardner et al., 1977) indicated that inhaled benzene was less toxic after intermittent than after continuous exposure, but the reverse occurred with nitrogen dioxide. Literature on aquatic species, based primarily on acute toxicity of

ammonia and metals, generally also indicated that relationships between time and concentration components of toxic exposures vary with chemicals (Brown et al., 1969; Ingersolland and Winner, 1982; Thurston et al., 1981). At least two major factors interact to yield such results. Some expression of average exposure concentration is typically used to compare regimens. Intermittent exposures then necessarily reach higher peak concentrations than do continuous ones to achieve the same average. Peak concentration could play a central role in toxicity when it (1) is a prime determinate of bioaccumulation (already discussed), or (2) exceeds a threshold concentration at a site of action (i.e., the lung or gill epithelium). On the other hand, recovery may occur during periods toxicant is absent as in intermittent exposures. Determinants of response probably include mechanism of toxicant action, status of detoxication systems, exposure concentration and duration, length of period between exposures, and route of exposure. Such factors should be considered in the context of temporal aspects of environmental occurrence of a chemical prior to recommendation of regulatory standards.

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Received July 16, 1984
Accepted October 16, 1984

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